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Interaction of *Helicobacter pylori* (Hp) and nonsteroidal anti-inflammatory drugs (NSAID) on gastric mucosa and risk of ulcerations

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Summary

Hp and NSAID are the most common pathogens in the stomach, but their interaction on gastro-duodenal mucosa has been little studied. Hp infection in humans does not interfere with NSAID-induced gastric ulcer healing by omeprazole, therefore, there is no rationale to eradicate the germ. Hp infection induces COX-2 expression resulting in excessive biosynthesis of gastroprotective prostaglandin (PG), which should in turn counteract NSAID-induced gastropathy and contribute to healing of existing ulcers. Some investigators claim that Hp infection acts synergistically with NSAID on ulcerogenesis and propose that Hp should be eradicated, particularly at the onset of long-term NSAID therapy.

Our studies in about 6500 dyspeptic patients undergoing upper endoscopy and ^{13}C-urea breath test revealed that about 70% of these patients are Hp positive and 31% of these develop gastro-duodenal ulcers. Of these ulcers, 66% were Hp positive and NSAID negative, 3% – NSAID positive and Hp negative, 8% were both Hp positive and NSAID positive, while 23% ulcers were Hp and NSAID negative. An evidence was obtained for negative interaction between Hp infection and NSAID on risk of gastro-duodenal ulcers suggesting that Hp may attenuate the peptic ulcerogenesis.

Our results support the concept 1) the interaction between Hp infection and NSAID on gastro-duodenal ulcerations is antagonistic, 2) the Hp and NSAID are independent risk factors for peptic ulcerations in humans, 3) there is no need for the Hp eradication in NSAID-treated patients, and 4) the rate of ulcer complications (hemorrhage and perforation) remains constant despite the decrease in Hp and ulcer prevalence.

key words: **Helicobacter pylori • aspirin • non-steroidal anti-inflammatory drugs • peptic ulcer**

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PATHOGENESIS OF GASTRIC ULCERS INDUCED BY HP INFECTION AND NSAID

Hp infection and NSAID use are generally considered as major pathogens in gastro-duodenal mucosa but the mechanism underlying the damage appears to be quite different. Hp infection initially induces acute and then chronic active mucosal inflammation (Bacterial gastritis – gastritis B) (Figure 1). The acquisition of Hp in the stomach requires penetration of bacteria through 'unstirred' layer of mucus/HCO_3^- covering the surface epithelium to reach their surface and to adhere directly to it [1]. This is facilitated by bacterial flagella allowing for mobility of Hp within the mucus to reach the surface of epithelial cells and to adhere to them *via* bacterial pseudopodia. Following adherence to surface epithelium Hp acts directly on the cells to 'inject' (by so called type IV secretion system [2–4]) cytotoxins, especially those encoded by *cytotoxin-associated gene (cagA)*, a marker for pathogenicity islands, containing various genes. Once CagA protein and other cytotoxins of Hp have been translocated into the mucosal cells, they result in the tyrosine transphosphorylation along with other cytosolic protein [2,4,5] and enhance the rate of host cell growth and induction of potent cytokines such as interleukin-8 (IL-8) *via* nuclear factor (NF) kappaB (NFκB) [2,4–6]. This Hp – host mucosal cell interaction resembles 'Trojan horse' for the host cell [5] because it causes not only the release of cytokines such as IL-8 involving MMK and NFκB [6] (Figure 2), but also results in direct damage to the host cells. In addition, CagA has been proposed to be an important risk factor for the development of peptic ulceration and even gastric cancer in Hp infected gastric mucosa [1,7]. The question continues to be contentious and studies on *cagA* and *vacA* as well as IL-1β polymorphism [8,9] may provide additional information regarding the link between Hp and ulcerogenesis, carcinogenesis and other gastroduodenal diseases. Furthermore, Hp in the stomach stimulates the release of leukocyte-chemoattractants [C5a, N-formyl peptides (fMLP), platelet activating factor (PAF), leukotriene B4 (LTB_4)], chemokins [IL_8, MCP-1, RANTES] and tumor necrosis factor alpha (TNFα) [9,10]. TNFα in turn upregulates the expression of selectins and their ligands (ICAM-1 and VCA-1) and integrin receptors [11]. These pro-adhesive factors are balanced by endogenous anti-adhesive substances including transforming growth factor alpha (TGFα) and nitric oxide (NO) induced by Hp from the infected mucosal and non-mucosal cells, including macrophages, leukocytes, miocytes etc.

Hp in gastric mucosa, mainly in gastric *antrum*, where this germ is usually localized, induces usually chronic active gastritis (*antritis*) with overexpression of COX-2. Similarly, the infection of the margin of the ulcer infected by Hp causes an over-expression of COX-2 without affecting COX-1 expression [12] and results in the over-expression of some growth factors such as TGFα and VEGF.

The excessive biosynthesis and release of 'cytoprotective' prostanoids, particularly prostaglandin (PG) E_2 (PGE_2) due to overexpression of COX-2 results from the activation of the whole arachidonate cascade starting from phospholipase-activated release of arachidonic acid from the phospholipid membrane to its transformations to PGG_2 and PGH_2 and finally to PGE_2, PGI_2, $PGF_{2\alpha}$, PGD_2 and its PGJ_2. PG released in excessive amounts acts *via* various membrane-bond receptors on target cells causing various physiological responses. These effects of PG limit the extent of mucosal damage and accelerate mucosal repair and ulcer healing *via* stimulation of angiogenesis, expression of proliferating

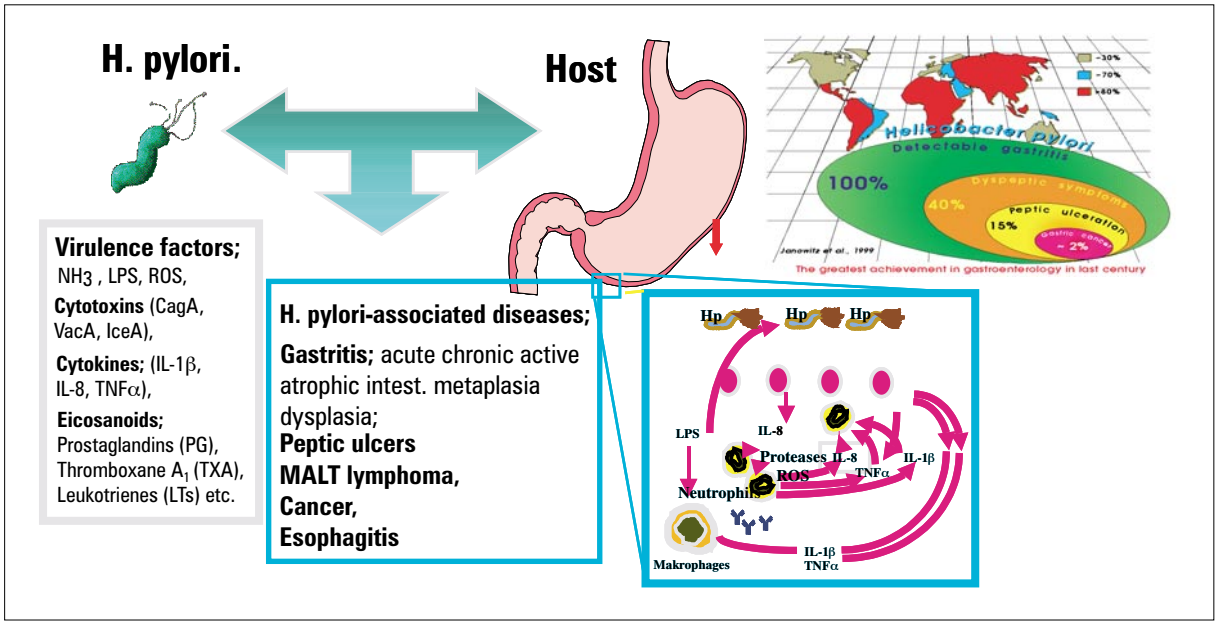


Figure 1. Hp-infection with world-wide distribution and Hp-induced induced gastritis, its virulence factors and mediators involved in the biological balance between Hp and the host.

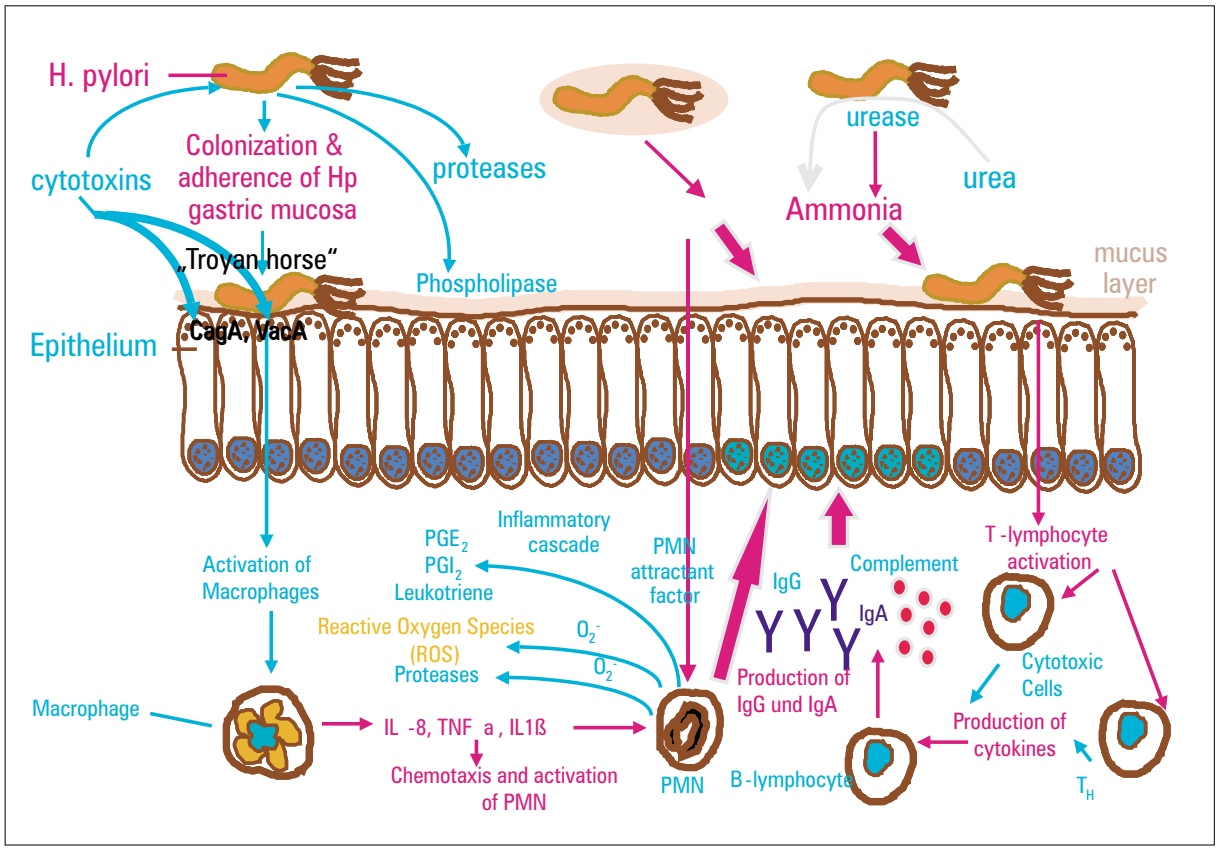


Figure 2. Mechanisms of Hp-induced gastritis. Hp acts as Trojan horse injecting cytotoxins to epithelial cells and intracellular events leading to expression, production and release of cytokines, especially interleukin-8 (IL-8), reactive oxygen species (ROS) and antibodies against Hp and its cytotoxins.

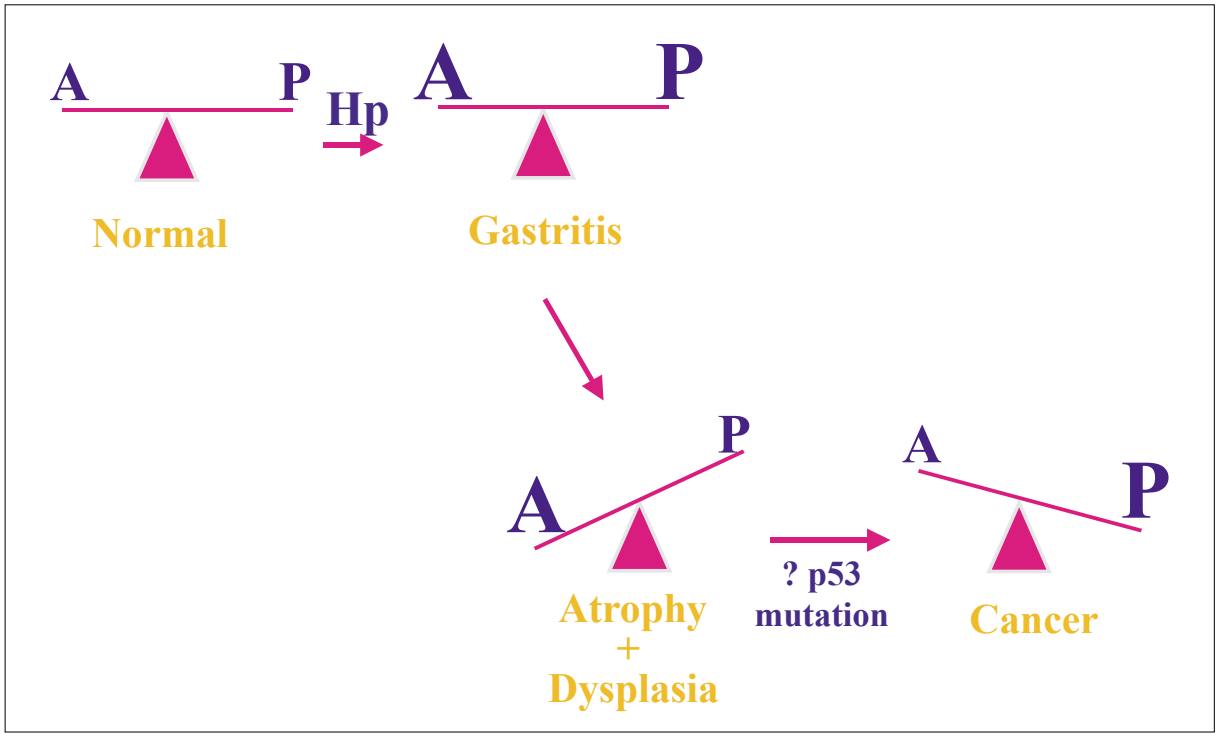


Figure 3. Effects of Hp infection on the balance between apoptosis and mucosal cell proliferation in chronic gastritis, atrophic gastritis and cancer (Mutation of P53 is also shown).

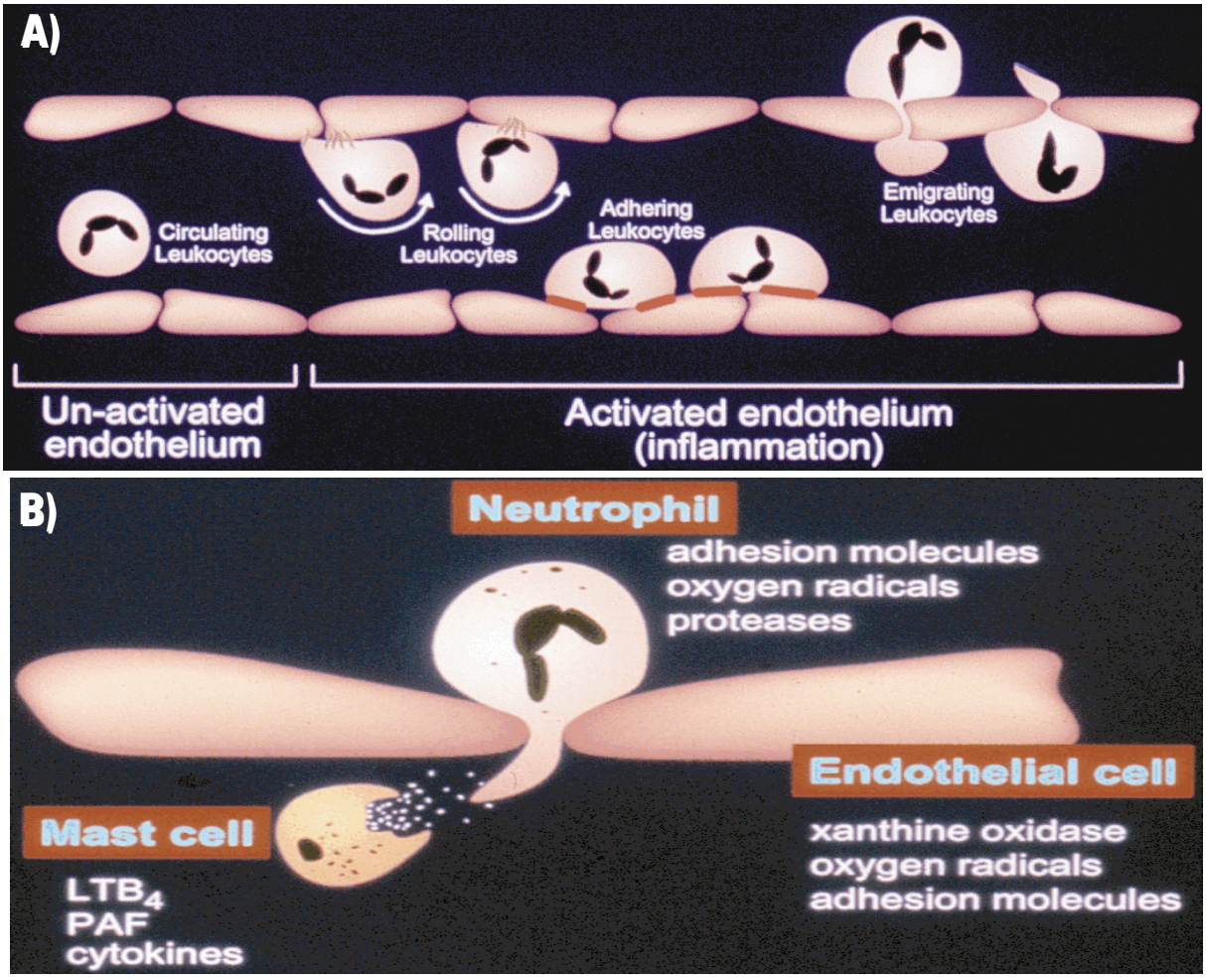


Figure 4. Local and systemic effects of NSAID resulting in activation of leukocytes, their interaction with endothelium leading to ischemia and reactive oxygen species and finally in formation of bleeding erosions and ulcer formation (Scheiman J.M., 1996).

factors such as transforming growth factor alpha (TGFα) and hepatocyte growth factor (HGF) [13], resulting in proliferation of mucosal cells to replace those damaged by Hp and repair of mucosal lesions [1].

It is of interest that Hp-induced gastritis is associated (at least at certain time periods after infection) with an increase in apoptosis attributed to the action of various products [ammonia, cytotoxin, lipopolysaccharides (LPS), endotoxins, leukocyte attractive factor etc.] released from the bacteria itself or released by the infected mucosa, (cytokins, interleukin 8, interleukin1β and others, especially TNFα) released either by Hp itself or by mucosal cells infected by this germ [13]. This is not always the case, and quite often hyperproliferation of mucosal cells (observed in chronically infected mucosa) may be a secondary phenomenon initiated by excessive cell loss. It is of interest that hyperproliferation of mucosal cells may involve also stimulation of apoptosis which under special conditions may eventually result in cancer development (Figure 3).

In contrast, the NSAID, present in acidic gastric content, exert their damaging action on gastric mucosa in two ways; 1) major systemic mechanism involving an inhibition of cyclooxygenases (COX) and 2) by local COX-independent mechanism (Figure 4) by breaking the gastric mucosal barrier, penetrating mucus layer to reach the surface of epithelial cells and diffuse into mucosal cells in acidic gastric lumen (pKa for aspirin is 3.5) by non-ionic diffusion. During this diffusion, the cells of surface epithelium loose their hydrophobicity and the ability to repel the polarized substances such as HCl, while aspirin and other acidic NSAID diffuse and accumulate within cells. Here they dissociate and being 'trapped' in their cytoplasm, they affect enzyme activity, uncouple oxidative phosphorylation and suppress the expression and production of heat shock proteins (HSP) that normally are responsible for cellular integrity [14]. The damaged surface epithelium swell and forms with exfoliated cells the 'mucoid cap', allowing the penetration of luminal H+ into the mucosa to release various inflammatory mediators and to damage the microvascular wall, increase its permeability and to decrease the mucosal blood flow [14-16] (Figure 5).

Another mechanism of local action of NSAID is the release from mucosal cells of TNFα, which unpregulates

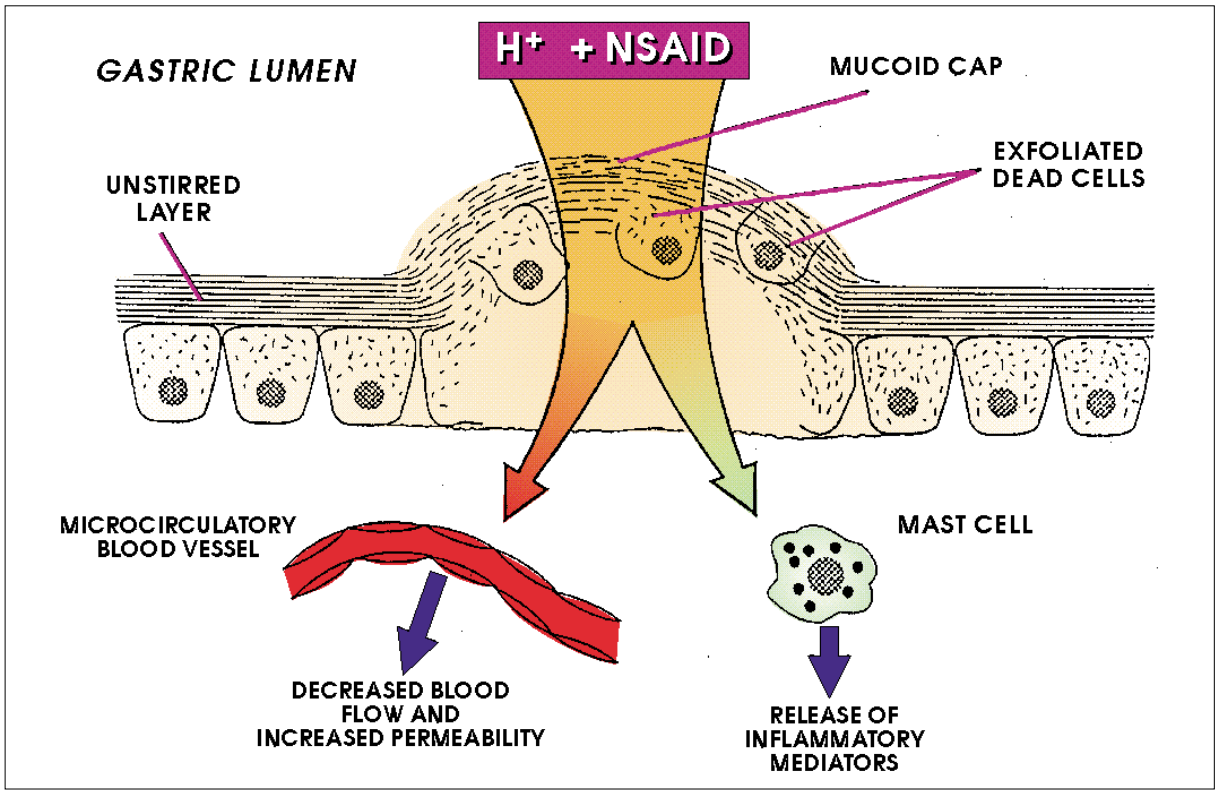


Figure 5. The dependence of NSAID-induced topical gastric mucosal damage upon gastric acidity (pH <3.5) and consequence of non-ionic diffusion of acidic NSAID into mucosal cells with damage of these cells, disturbance of microcirculation and activation of mast cells releasing inflammatory mediators (LTB4, IL-1β, TNFα).

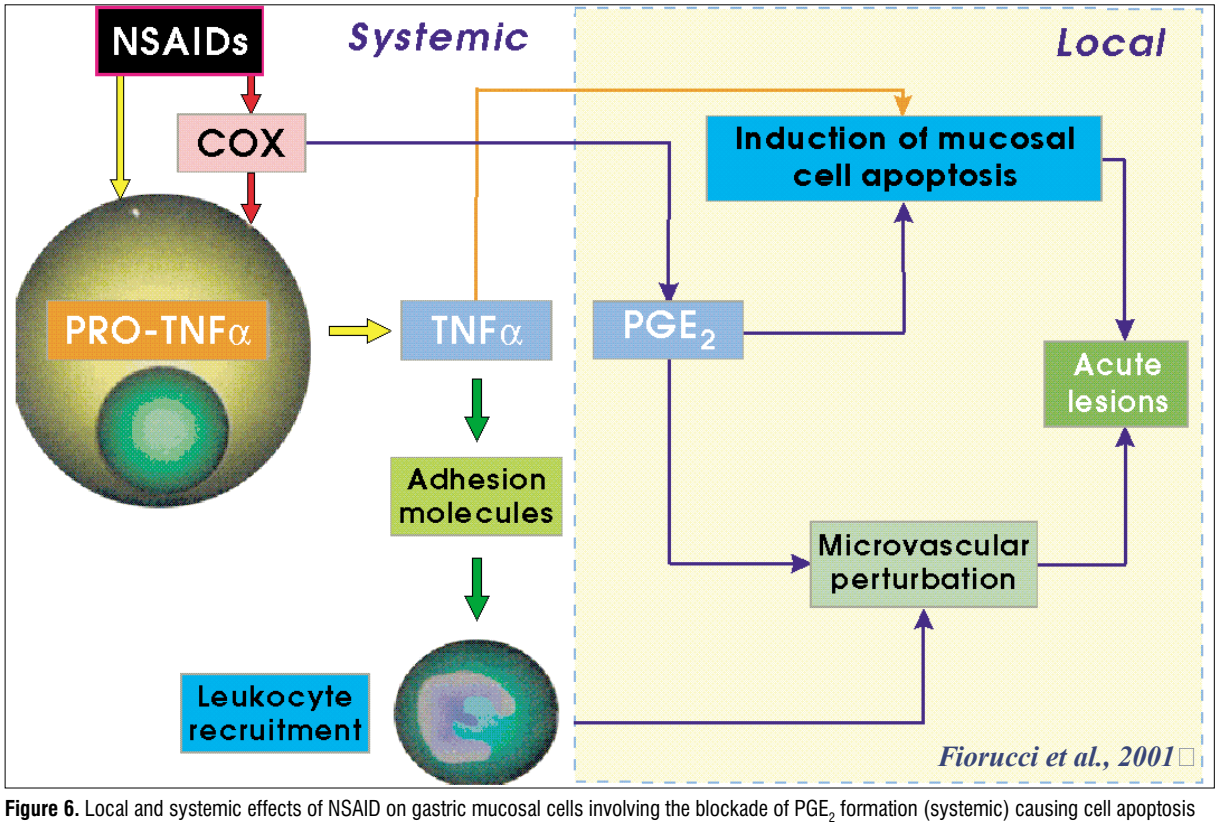


Figure 6. Local and systemic effects of NSAID on gastric mucosal cells involving the blockade of PGE_2 formation (systemic) causing cell apoptosis and microvascular perturbation and release of TNFα contributing to leukocyte recruitment and mucosal damage (Fiorucci et al, 1999).

adhesion molecules and activates neutrophils, leading to infiltration of gastric mucosa, reduction in mucosal blood flow and the formation of acute erosions and ulceration (Figure 6). TNFα also activates proapoptotic caspases *via* stimulation of nuclear transcription factor kappa B (NF-κB) leading to protection of P53-DNA repair mechanism and enhancement of apoptotic pathway (apoptosis is programmed cell death without surrounding inflammation). NSAID administration causes an increase in serum levels and mucosal TNFα leading to excessive mucosal cell loss and development of peptic ulceration during acute phase and epithelium cell atrophy with subsequent atrophic gastritis, termed gastritis C (chemical).

The induction of mucosal cell apoptosis by NSAID is accompanied by the fall in PG biosynthesis due to the suppression of COX by NSAID and this results in microcirculation disturbance, augmentation of activity of neutrophils and interaction of activated neutrophils with damaged endothelium leading to obstruction of capillaries with formation of 'white' thrombi originally described by Kitahora and Guth [17].

ROLE OF PROSTAGLANDIN (PG) IN GASTRIC MUCOSAL INTEGRITY

Almost all mammalian cells contain COX, the first enzyme in the pathway converting membrane phosholipids originating arachidonic acid to PGE_2, PGD_2, PGI_2, PGJ_2 and TX_2 [18]. COX exists in at least two district isoforms, COX-1 and COX-2. COX-1 is considered a constitutive enzyme present in almost all cell types though under certain conditions it can also be induced [19]. COX-2 may be normally expressed and active in certain normal cells such as *mascula densa* on kidney, uterus and even in endothelial cells but in gastrointestinal mucosa it is not normally present unless infected e.g. with Hp, ulcerated (gastroduodenal ulcers or ulcerative colitis) or involved in cancerogenesis. The expression of COX-1 in gastric mucosa is very rapid [20] with 1–2 h upon mucosal irritation and following blockade of cyclooxygenase, possibly to compensate for the tissue loss of PG. Wallace et al. [21] claim that the inhibition of either COX-1 or COX-2 does not induce mucosal damage in rats but their combination is required to cause this damage. Most important COX-2 expression is excessive in the inflammation, i.e. in gastric mucosa exposed to stress ischemia/reperfusion [22,23] caused by Hp or in colonic mucosa involved in ulcerative colitis in dysplasia or neoplasia. Other prostanoids such as PGI_2 produced mostly by endothelium acts *via* IP receptors that prevent platelet aggregation to cause vasodilatation, natriuresis and gastric acid inhibition. $PGE_{2\alpha}$ acts *via* FP receptors to induce uterus contraction, vasoconstriction, bronchospasm and reduction in gastric acid secretion. PGD_2 uses DP receptors to increase gastric and renal blood flow, to inhibit gastric H^+ secretion and platelet aggregation. PGD_2 can be transformed to PGJ_2 that is the known stimulus of tissue repair and healing to induce NO synthase. TXA_2 acts *via* TP receptors to increase plasma Ca^{2+} level resulting in platelet aggregation and potent vasoconstriction. The biological effects of COX products, PG, are mediated by specific membrane receptors termed *EP receptors* (EP_1, EP_2, EP_3, EP_4) that are bound to membrane G-proteins being linked to a different intracellular signal transduction pathway [24]. Binding to EP_1 results in intracellular release of triphosphate inositol (IP_3) and diacylglycerol (DAG), to EP_2 and EP_4 – activates adenylcyclase-cyclic adenosine monophosphate (cAMP) and to EP_3 – inhibits adenylcyclase-cAMP system.

In contrast to COX-1, COX-2 is overexpressed in inflammatory tissue being strictly an enzyme inducible by cytokines, growth factors, including gastrin, and tumor promoters [24] (see Figure 3). COX-2 is responsible for excessive production of PG associated with inflammation, and this is due to a special large channel allowing arachidonic acid to remain longer and in close vicinity to an active center of the enzyme than in COX-2 (Figure 9) while COX-1 is involved in the production of PG to maintain gastrointestinal mucosal integrity [25].

The regulation of PG biosynthesis is complex and depends not only on expression of COX-1 or COX-2 but also upon the availability of their substrate, arachidonic acid, released by phospholipases (PLA), particularly PLA_2, from the membrane phospholipids. Cytosolic PLA_2 ($cPLA_2$) is calcium-dependent and selective for phospholipids containing arachidonic acid. Secretory PLA_2 ($sPLA_2$), exists in five distinct isoforms [26] and one of them known as inflammatory-type $sPLA_2$ is highly expressed in inflamed tissues such as Hp-induced gastritis through the action of proinflammatory cytokines, especially TNFα [24].

Since the function of prostanoids involves various receptor sites, mice with targeted gene disruption (knock-out mice) have been used to identify the real function of each prostanoid [24,26,27]. Physiologically, PGE_2 is responsible for increasing gastric (GBF), renal blood flow (RBF) and vasodilation, inhibition of gastric acid secretion and natriuresis. PGD_2 raises RBF and inhibits gastric acid secretion, PGI_2 inhibits platelet aggregation and gastric acid secretion. $PGF_{2\alpha}$ is involved in the increase in uterine contraction and vaso- or bronchoconstriction. Thromboxane A_2 is responsible for enhanced platelet aggregation and vasoconstriction.

Most of conventional NSAID inhibit both COX-1 and COX-2 with predominant action on COX-1 and only a small effect on COX-2. Novel agents, however, inhibit COX-1 but may also preferentially suppress COX-2 and novel agents termed COX-2 inhibitors cause selective suppression of COX2 activity. The marketing of these agents and their widespread use is supported by numerous clinical trials indicating their significantly less gastrotoxicity and smaller side-effects from the gastrointestinal tract. However, despite their relative selectivity most available NSAID inhibit also COX-1 and still to some extent are gastrotoxic. They also delay the healing of preexisting lesions of gastrointestinal mucosa and they can induce hypertension and even myocardial infarction when supplied without aspirin to block COX-1 and prevent vasoconstriction [28]. In contrast to con-

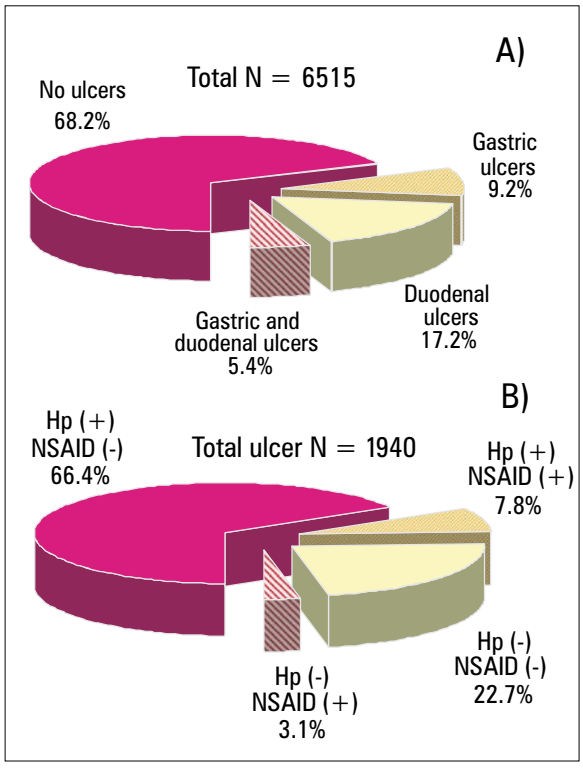


Figure 7. Prevalence of gastro-duodenal ulcers (N=1898) in dyspeptic patients infected with Hp. Using NSAID, both of them or neither of them. Only Hp infection but not NSAID was effective in significant increase in ulcer prevalence (A), while 66.4% of ulcer patients show both Hp infection and NSAID use, there is about 22.7% without ethiological factor, hence then are termed 'idiopathic' ulcer (B) [32]

ventional NSAID, selective COX-2 inhibitors fail to alter the PG biosynthesis under steady state conditions but suppress the generation of PG in gastric mucosa infected with Hp at the ulcer area, especially at its edge, a crucial area for the healing of the gastric or duodenal ulcer.

PG produced by both COX-1 and COX-2 are responsible for the homeostasis of gastro-duodenal mucosa and maintenance of its integrity by stimulating mucus/HCO_3^- secretion that provides a protective blanket of 'unstirred' layer covering mucosal surface, increased mucosal blood flow, enhanced epithelial cell migration, restitution and proliferation and activation of mucosal immunocyte function [16].

There is little doubt that Hp stimulates mucosal PG biosynthesis (1) possibly through increased COX-2 expression by growth factors such as gastrin [12,29,30]. PG are the major endogenous substances contributing

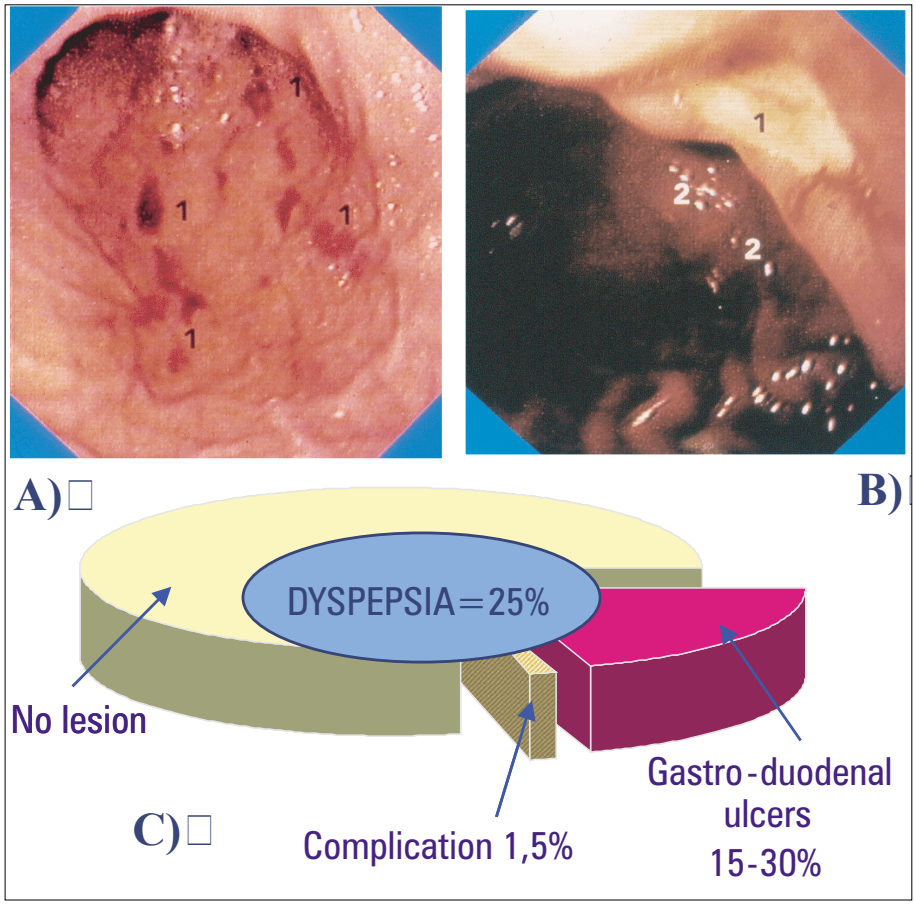


Figure 8. Acute bleeding erosions (A) and chronic gastric ulceration (B) in patients using single doses of aspirin. Overall occurrence of dyspepsia, gastro-duodenal ulcers and their complications in NSAID users (C) [1].

to mucosal integrity and are produced in large amounts by COX-2 just around gastric ulceration [12], resulting in local expression of other protective substances, that also may contribute to ulcer healing. In addition to growth factors including gastrin, epidermal growth factor (EGF), transforming growth factor alpha (TGFα), basic fibroblast growth factor (bFGF) and nitric oxide (NO) are co-expressed by COX-2 within inflamed tissue e.g. at the ulcer edge. An inducible NO synthase (iNOS) also may be implicated in gastric ulcerogenesis [12].

These observations led to the speculation that the PG derived from the gastric mucosa infected with Hp might play a protective function against the NSAID-induced gastric injury [31–35] but this has been questioned because eradication of Hp failed to impair healing of NSAID-induced gastric lesions using potent inhibitors of gastric acid secretion such as omeprazole [31]. On the other hand, Hp infection, despite overexpressing COX-2 and releasing excessive PG biosynthesis, by itself, is capable of inducing ulcer formation and about 60% of these ulcers develop due to this infection. According to our experience less than 10% could be attributed to NSAID [32] (Figure 7). Following Hp eradication, the NSAID ulcers do not heal at higher rate than in those with preserved Hp infection [34]. The importance of PG originating from Hp infection is, however, difficult to assess in Hp infected NSAID users because these drugs are potent inhibitors of both COX-1, COX-2 so it is unknown what degree of PG deficiency develops in gastric mucosa though it was found that these drugs despite inhibition of COX activity and formation of acute and chronic gastric ulcerations (Figure 8) cause enhanced expression of COX-2 in the Hp infected stomach [12,29].

EPITHELIUM DAMAGE, LYMPHOCYTE INVOLVEMENT AND NEUTROPHIL-MEDIATED MUCOSAL INJURY BY HP AND NSAID

Colonization of gastric mucosa with Hp and its infection results from the ability of this germ to remain viable within the mucus covering this mucosa despite the hostile acid environment of gastric lumen that usually kills most of the ingested bacteria and 'sterilizes' gastric content. The potent urease activity of Hp has been successfully exploited for testing the presence of active Hp infection using ^{13}C-urea breath test. Urease (UBT) represents a well-defined gastric colonization factor for this germ through formation by alkaline ammonia (NH_3) a 'blanket' around the bacteria that neutralizes and counteracts the noxious effects of gastric luminal HCl. The urease encoding gene present in Hp genome, termed *urel* has been shown to be pH-sensitive and to function as urea channel [35,36] explaining how the viability of this particular bacteria can be preserved in acid environment in gastric lumen. The deletion of urel abolishes Hp acid resistance, confirming that urease activity, that is strongly activated at lower pH, is a prerequisite of bacteria viability and growth in acidic gastric environment.

Another toxic substances produced by most of Hp are lipopolysaccharides (LPS) that are similar blood group antigens such as Lewis X (Le^X) or Lewis Y (Le^Y). These LPS of Hp have been associated with host epithelial cells and implicated in Hp-induced gastric pathology [37,38]. The blood group antigen binding adhesin (BabA) of Hp encoded by $babA_2$ of this germ has been associated with Hp infection outcome and in combination with *cagA* and *vacAS1* has been shown to strongly correlate with the development of peptic ulcers or gastric cancer as compared with *CagA* and *vacAS1* alone [10]. According to our experience, small doses of Hp-LPS may induce gastroprotection against various topical irritants, accompanied by increased microcirculation, while larger doses have opposite effects, namely, they decrease both systemic blood pressure and the fall of gastric blood flow causing mild superficial injury to gastric mucosa and prolong the healing of chronic gastric ulcers *via* induction and activation of COX-2 and iNOS with excessive release of prostanoids and other products of arachidonate metabolism as well as NO [37]. It is of interest that the gastroprotective effects include the cerebral centers because the intra-cerebral administration of this LPS resulted in gastroprotection similar to that applied peripherally but disappearing after inactivation of afferent sensory nerves. In brief, that the presence of Hp in the stomach acts on the gastric mucosa through activation of brain-gut axis, the release of NO probably due to the release of calcitonin gene-related peptide (CGRP) and other sensory neuropeptide. Evidence shows that endogenous opiates may also contribute to the protection involving brain-gut axis because blockade of mμ-receptors with naloxone reverses gastroprotection afforded by peripheral or central LPS. Thus, Hp confined to the gastric mucosa appears to involve the peripheral neural pathways as well as cerebral centers and brain-gut axis.

GASTRIC IMMUNE RESPONSE

Hp infection also induces a potent immune response in the host [10,39]. Upon bacteria stimulation, native T-helper (Th) precursor cells differentiate into Th_1 that are associated with cytokine (IL-2 and ITFγ) production and cell-mediated immune response and into Th_2 that promote activation of B-lymphocytes and humoral immunity response including the release of various cytokines (IL-4, IL-5, IL-6 and IL-10) [8,9]. Since Hp is a typical extracellular pathogen and non-invasive by nature but associated with exuberant humoral response, it is expected to induce Th_2 response, but paradoxically this germ induces antigen specific T-cell clones in the gastric mucosa, producing high level of ITFγ and IL-4, reflecting Th_1-type response (40). Hp also stimulates IL-12 which promotes Th_1 differentiation suggesting that the bacterial gastritis (gastritis B) involves both Th_1 and Th_2 immune responses and perpetuates gastric inflammation from an acute superficial to deeper active chronic gastritis and then gastric atrophy [7]. Infection with Hp strains producing cytotoxin encoded by *cagA, vacA, iceA* and IL-8 released from the damage mucosal cells lead to intense atrophic gastritis [39,40]. Host cytokine polymorphisms such as that of IL-1 have been associated with an increasing risk of gastric carcinogenesis [9,10,41], but this awaits confirmation.

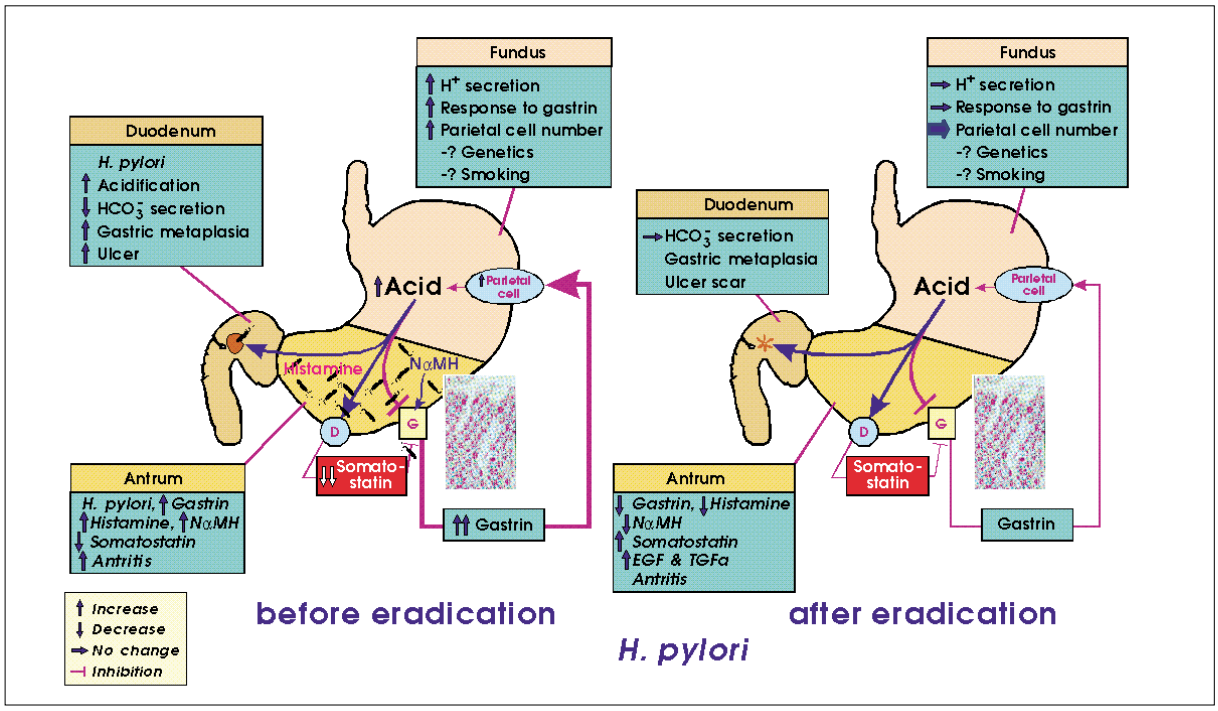


Figure 9. The secretory, gastrinic and mucosal profile in Hp infected duodenal ulcer patients before and after the eradication of Hp.

An important feature of Hp induced gastritis is an alteration in epithelial cell apoptosis [41–43] with compensatory cell proliferation possibly mediated, at least in part, by excessive expression of growth factors [43]. Signaling through Fas death receptor has been postulated to mediate apoptosis either *via* activation of these receptors directly on gastric epithelial cells and/or through the production of cytokines such as TNFα [44]. Enhanced rate of apoptosis and subsequent widespread mucosal cell death may accelerate progression of atrophic gastritis with concomitant increase in the risk for distal gastric cancer, while reduced apoptosis and cell loss leading to heightened retention of mutagenized cells may also predispose to gastric cancerogenesis. Th_1 lymphocyte derived cytokines such as IFNγ are synergistic with Hp to induce Fas ligand (FasL) in epithelial cells, resulting in Hp-induced apoptosis [45].

In addition to direct mucosal cell damage by Hp and its mediators, there is increasing evidence for Hp producing a neutrophil activation factor [46] resulting in mucosal infiltration involving also the release of IL-8 and formation of reactive oxygen species [46,47]. NSAID also increase neutrophil activation and adherence to endothelium to liberate reactive oxygen species, proteases with obstruction of blood flow in the gastric mucosa vessels [13,17,48] neutrophils have also been implicated in the pathogenesis of NSAID-induced gastropathy [15] and the combination with Hp infection was suggested to aggravate NSAID-induced mucosal injury due to enhancement of production of IL-8 and to release of oxygen free radicals. Thus, the interaction of Hp and NSAID was reported to activate gastric neutrophils and release reactive oxygen species, resulting in increased incidence of mucosal lesions in Hp-NSAID users as compared with non-infected NSAID users only.

GASTRIC ACID SECRETION IN HP AND/OR NSAID AFFECTED STOMACH

Intragastric acidity and the gastric acid secretory activity have been reported to be altered by Hp infection and by NSAID [17,43,49]. Acute Hp infection in humans was reported to cause transient hypochlorhydria due to direct action of Hp-derived inhibitory restoration within few months of gastric acidity and secretory activity [1], particularly in *antrum*-dominated chronic active gastritis (antritis) [9]. This is accompanied by increased gastrin release due to reduction in somatostatin and impairment of paracrine inhibitory action on the G-cells [1]. These functional secretory changes caused by antral inflammation with Hp, disappear upon the eradication of Hp using pantoprazol-based one-week triple therapy (Figure 9).

However, an extension of Hp infection to gastric *corpus* and/or production of autoantibodies against parietal cells that bind and inactivate H^+-K^+-ATP-ase on luminal surface of the acid secreting cells may result in gastric atrophy with permanent achlorhydria [1]. As gastric mucosal infection may last for decades and the bacteria colonize predominantly antral portion of the stomach (*antritis*) or the *corpus* of the stomach (*corpusitis*) or both, pangastritis with mucosal atrophy may be accompanied by a severe decrease in gastric secretory activity, the alteration in gastrin release (decrease in atrophic antritis and increase in corpusitis) and enhanced susceptibility to develop intestinal metaplasia, dysplasia and gastric ulcer or cancerogenesis (Figure 9).

In contrast to Hp, NSAID use is expected to raise gastric acid secretion, because of their ability to inhibit PG biosynthesis but this is usually not the case because in long-term users of NSAID the breaking of gastric mucosal barrier and reduction in blood flow occur [17]. This results in the damage of surface epithelium [50] and followed by back-diffusion of luminal acid with subsequent reduction in gastric acid secretion and increased luminal pH but this is not always the case due to inhibition of somatostatin by NSAID (Ligumsky et al, 1983). Nevertheless, luminal acid (pH <3.5) is required for acidic NSAID, such as aspirin to penetrate the mucosa and its damage (see Figure 6). NSAID suppress natural cellular defence mechanism involving the release of heat shock proteins (HSP) and enhanced apoptosis [14].

Because mucosal damage by NSAID in gastric lumen depends upon its pH and the capability of oxyntic mucosa to secrete acid, the pH infection with a different pattern of gastritis from mild superficial to atrophic gastritis greatly influences the NSAID-mucosal damage in Hp infected stomach. It is of interest that Hp infected patients with antral-predominant gastritis (*antritis*) [9] are prone to develop duodenal ulcers, while the use of NSAID usually results in *corpus* predominant gastritis (*corpusitis*) or *corpus* atrophy. Rheumatoid patients usually exhibit lower gastric acid secretion than healthy controls [51] and this is possible due to gastric atrophy including oxyntic gland area. Better tolerance of long NSAID user in Hp infected patients could be secondary to hypochlorhydria resulting from corpus gastritis (*corpusitis*).

NSAID-HP INTERACTION IN THE RISK OF GASTRO-DUODENAL ULCERATIONS AND THEIR COMPLICATIONS

The effects of Hp infection on NSAID-provoked ulcer development and its complication, particularly bleeding, appears to be affected by the concomitant use of a potent gastric acid suppressant such as proton pump inhibitors (PPI). Hp was found to increase the risk for gastro-duodenal ulcers in patients who do not use PPI [52], suggesting that mucosal damaging action of Hp may be overcome by acid inhibition. On the other hand, active Hp infection is known to increase gastric inhibitory efficacy of PPI due to an increased number of active proton pumps in the Hp infected mucosa [53]. In large trials ASTRONAUT or OMNIUM carried out by Hawkey abd coworkers [31,54] to compare the efficacy in NSAID-induced ulcers by gastric inhibitors (omeprazole vs. ranitidine) and by misoprostol vs. omeprazole), ulcer relapse at 6 months occurred in 75% in Hp positive and only in 60% in Hp negative patients. This suggests that Hp in the absence of gastric acid (due to administration of potent gastric inhibitors) appears to increase the NSAID-related ulcers, while opposite effects were observed in the stomach with acid suppression. The results of the ASTRONAUT and OMNIUM [31,54] are difficult to interpret regarding the interaction of NSAID and Hp infection in gastro-duodenal mucosa because of the above-mentioned reduction of the ulcer risk by PPI in NSAID-induced gastric ulceration. Therefore, the assessment of impact of Hp on NSAID-induced ulcerogenesis requires further studies in patients not taking PPI or other potent gastric inhibitors.

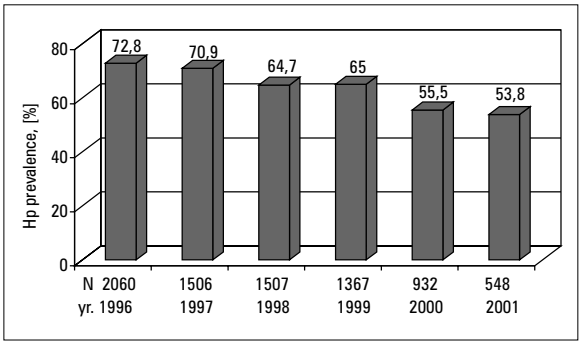


Figure 10. The prevalence of Hp infection in dyspeptic patients (total N=7920) in years 1996 to 2001 in Polish population [56].

The problem of the impact of Hp and NSAID on the risk of gastro-duodenal complications could be solved using large number of patients entering the trial with gastro-duodenal ulcers with or without Hp infection and with or without NSAID use to find out whether any of the above combination of ulcerogens could affect in a positive or negative way or not at all the occurrence and the healing rate of gastro-duodeenal ulcers and their complications.

Our clinical studies [32] included 6515 dyspeptic subjects, the world largest number of patients examined for Hp infection with ^{13}C-urea breath test (UBT) and gastro-duodenal ulcers with endoscopy, who entered consecutively into our trial in 1996-2001. The Hp prevalence averaged about 70% and about 30% of tested subjects showed endoscopically gastric and/or duodenal ulcerations and among these active ulcer patients, 66.4% were Hp positive, 7.8% were Hp infected NSAID users, 3.1% had ulcers associated with both Hp and NSAID use and finally 22.7% were Hp negative and non-users of NSAID. The latter group could be defined as 'idiopathic'ulcers as no evidence for any major known ulcerogens such as Hp infection or NSAID could be found (Figure 10).

This group is of particular interest because the contribution of such idiopathic ulcers gradually increased in the last years, at least in the Polish population, while the overall occurrence of peptic ulceration during the same period declined gradually from 44% in 1996 to 7.7% in 2001 and it was accompanied by steady reduction in Hp prevalence in the Polish population from about 74% in 1996 to 54% in 2001. NSAID were used by about 10% of study patients throughout the study period. The etiological factors responsible for these non-Hp, non-NSAID ulcers are unknown, but neither smoking nor family link (genetic factor) was found to be responsible as they were similar to those in ulcers associated with Hp infection alone, with NSAID use alone, Hp alone and with Hp plus NSAID use. Physiological stress and diet [55] have been suggested to contribute to the

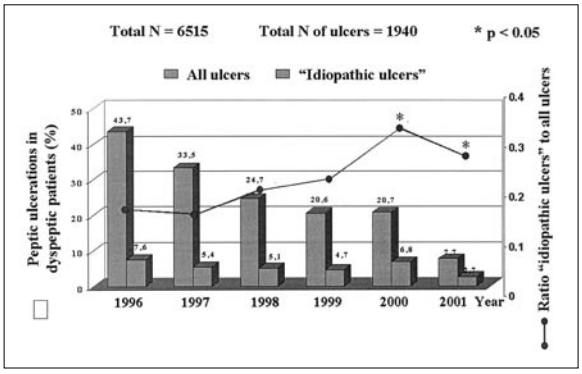
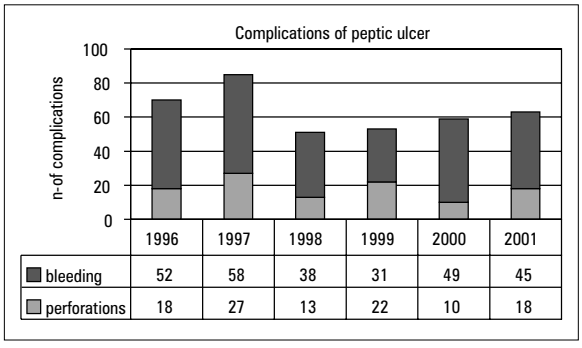


Figure 11. The occurrence of gastroduodenal ulcerations and the ratio of idiopathic ulcers to all ulcers (N=1940) among 6515 dyspeptic patients subjected to endoscopy between 1996 to 2001 [56].



	1996	1997	1998	1999	2000	2001
bleeding	52	58	38	31	49	45
perforations	18	27	13	22	10	18

Figure 12. Complications of peptic ulcers, bleeding and perforations in one larger surgical unit between 1996 to 2001 [56].

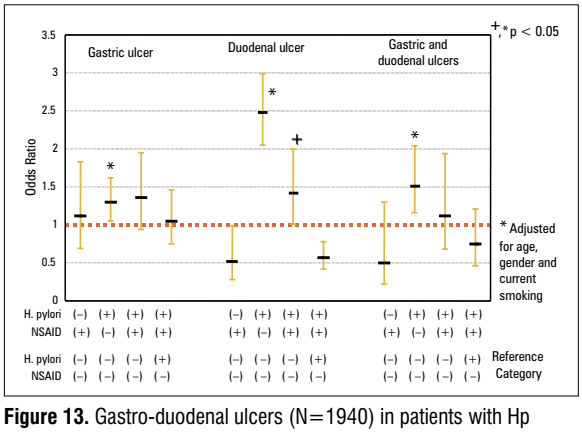


Figure 13. Gastro-duodenal ulcers (N=1940) in patients with Hp infection, with Hp infection plus NSAID use, with NSAID use only and without Hp plus NSAID (idiopathic ulcers). Multivariate regression analysis shows that only Hp alone or combined with NSAID increases the ulcer risk when compared to that in patients without Hp and/or NSAID [32].

pathogenesis of non-Hp, non-NSAID ulcers but this issue needs confirmation. It is of interest that despite the fall in Hp prevalence and the occurrence rate of gastro-duodenal ulcers, their complication such as hemorrhages and perforation in the same population sample actually remained constant (Figure 11). The reason for the sustained complications in gastro-duodenal ulcerations is not clear but the increase use of NSAID, especially at older patients could account for this phenomenon. It is of interest that the rate of complications such as hemorrhage and perforations remained relatively constant during the study period probably due to increased use of NSAID and the appearance of larger number of idiopathic ulcerations (Figure 12) [56].

Our study does not confirm or support the results recently published by Huang et al. [33], who based their conclusions on meta-analysis of 16 studies from various countries showing that Hp infected NSAID users have higher proportion of peptic ulcers than those with Hp alone or NSAID alone (Figure 13). We found no evidence for such synergism between Hp and NSAID possibly due to the fact that NSAID may directly suppress the Hp growth and activity of Hp resulting in elimination or, at least, limitation of contribution to gastroduodenal ulcerogenesis. Furthermore, according to Huang et al. [33] no peptic ulceration was observed in non-Hp, non-NSAID patients, while a study of Xia et al. [57] in a similar number of symptomatic patients (N=8344) entering the trial found a similar proportion of Hp associated ulcers (66%) or associated with Hp plus NSAID (8.5%) also revealing that about 17% were non-Hp, non-NSAID users in their series, emphasizing that these idiopathic ulcers have district clinical and endoscopic characteristics. Thus, unlike in experimental animals, Hp infection in humans taking NSAID does not significantly affect peptic ulcerogenesis, but ulcers may develop also without infection of Hp or NSAID and these ulcer patients require special care to prevent ulcer complications. Thus, Hp contributes to increased ulcer risk when acting alone so do also NSAID but no evidence for the synergism between these two types of ulcerogens was found in our well-controlled series. Hp-related to overexpression of COX-2 and excessive increase in mucosal generation of prostanoids appear to counteract the ulcerogenic efficacy of NSAID despite their ability to block COX-2 and suppression of prostanoid biosynthesis.

CONCLUSIONS

1. According to our data, there is no evidence for the synergistic interaction between Hp infection and NSAID as claimed by Huang et al. [33].

2. While about 70% of ulcers in dyspeptic patients might be attributed to Hp infection, both the Hp prevalence and ulcer occurrence show a tendency to decline in the last few years when the diagnosis of Hp and eradication therapy are a common practice.

3. A significant proportion of all ulcers (about 23%) appear to be non-Hp, non-NSAID (idiopathic) peptic lesions.

4. It is of interest that ulcer complications such as bleeding and perforation remain relatively constant over the last few years despite the reduction in Hp prevalence and the occurrence of peptic ulcers and this

could be explained by increased use of NSAID and the appearance of idiopathic ulcers.

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